6.4 kb

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Purpose: To amplify 6.4 kb and 8.0 kb from plasmid

used F + R (non d U) primers

50 µl Rx 200 µM dNTP each cycling: 94°, 1'

1.4 µM primers

2 mM Mg used buffer B (94°, 30" / 60°, 45" / 72°, 3') 25

Template ?

1 µl enzyme pre mixed (1 : 0.01)

spared enough premix for 20 Rx:

6.4 kb:

all done in duplicate.

included purified prep at a known concentration } can tried 50 pg + 100 pg (log 50) just one.

miniprepped, unknown concentration (from the amount of colonies in 1/100 dilution) Conc should be quite high in the miniprep. diluted to 50 µl } used .5 µl and 1 µl

plasmid - picked a single isolated colony directly into the reaction mix containing all the rest of the rxn done in duplicate

8.0

no purified stuff available

miniprep - unknown conc } lot of colonies from 1/100 → 25/µl dilution } .5 and 1 µl (out of 50 µl from 1.5 ml culture)

plasmid - 2, one in each - done in duplicate

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ssed & Understo d by me,	Date 1/9/95	Invented by	Date 1/5/95
		Recorded by K. Sikaroon	

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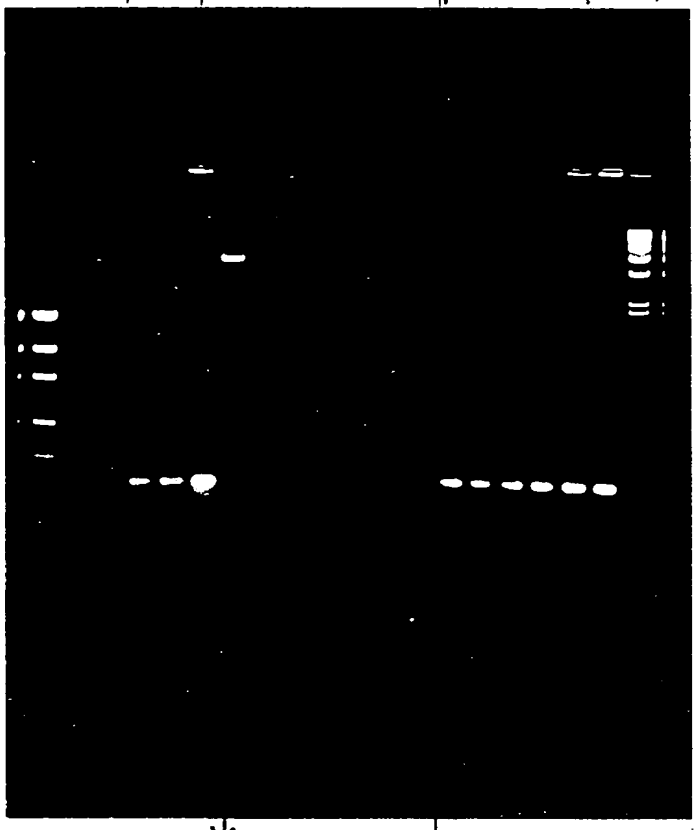

Results:

- 6.4 kb got amplified with lot of mispriming
- Taq 5U gave good amplification
- nothing to be seen in colonies, lot of stuff stuck up in the well
- Same time with both 6 kb + 8 kb
- not a good way to go no lysis at all,
- whenever there was no product lot of primer dimers
- amount of primers to be found amongst.

* check alternate cycling conditions to get rid of mispriming
** lysis in PK and gel workup has to be checked next
** make 6.4 kb to work first

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Witnessed & Understood by me,	Dat	Invented by	Dat
		Recorded by A. Lukaszewski	1/9/98